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Award Number: DAMD17-00-1-0514

TITLE: Volume-Stabilized Intravascular Microbubbles for Circulatory Transport of Oxygen and Carbon Dioxide: A Field-Usable Concept

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REPORT DATE: September 2001

TYPE OF REPORT: Final

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 074-0188

1. AGENCY USE ONLY (Leave blank)	2. REPORT DATE	3. REPORT TYPE AND DATES COVERED
	September 2001	Final (1 May 00 - 31 Aug 01)

4. TITLE AND SUBTITLE
Volume-Stabilized Intravascular Microbubbles for Circulatory Transport of Oxygen and Carbon Dioxide: A Field-Usable Concept

5. FUNDING NUMBERS
DAMD17-00-1-0514

6. AUTHOR(S)
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8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)

U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSORING / MONITORING AGENCY REPORT NUMBER

11. SUPPLEMENTARY NOTES

12a. DISTRIBUTION / AVAILABILITY STATEMENT
Distribution authorized to U.S. Government agencies only (proprietary information, Sep 01). Other requests for this document shall be referred to U.S. Army Medical Research and Materiel Command, 504 Scott Street, Fort Detrick, Maryland 21702-5012.

12b. DISTRIBUTION CODE

13. ABSTRACT *(Maximum 200 Words)*

Intravascular microbubbles generated by i.v. infusion of a 2% dodecafluoropentane emulsion (DDFPe) transports physiologically significant amounts of oxygen in oxygen breathing rats (1). The present study explores the question whether DDFPe treatment can sustain life during air breathing in severely erythrocyte depleted pigs. Anesthetized pigs were bled while given volume replacement with 6% dextran in lactated Ringer's solution. Artificial ventilation and/or oxygen admixture (<4%) to the inspired air maintained PaO_2 in the normoxic range (90-110 mm Hg). Control animals (n=6) received emulsion blank in addition to the plasma expander. They died at a hemoglobin level of 3.0 g/100 ml. The experimental animals received 0.7 ml DDFPe/kg body weight in an i.v. infusion lasting for ~190 min of the 260 min long exsanguination period. These animals were observed for more than one hour at hemoglobin levels averaging 2.1 g/100 ml. They retained a normal $P\bar{V}O_2$ and acid-base status indicating adequate tissue oxygenation and arterial blood pressures remained well above shock level. We conclude that DDFP derived microbubbles hold promise as a very effective erythrocyte substitute for circulatory oxygen transport in situations such as combat casualty care. Hence, the treatment should be tested in a circulatory shock model.

14. SUBJECT TERMS
erythrocyte substitute, intravascular microbubbles, combat-casualty care, dodecafluoropentane emulsion, circulatory oxygen transport, blood loss, hypoxia

15. NUMBER OF PAGES
26

16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT
Unclassified	Unclassified	Unclassified	Unlimited

NSN 7540-01-280-5500

Standard Form 298 (Rev. 2-89)
Prescribed by ANSI Std. Z39-18
298-102

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INTRODUCTION

This project aimed at studying the feasibility of a new treatment modality for erythrocyte depletion due to severe blood loss. We have earlier demonstrated that treatment with intravascular microbubbles consisting of dodecafluoropentane (DDFP) gas can, combined with oxygen breathing, provide life-sustaining oxygen transport in erythrocyte depleted rats (1, 2). The present work aimed at testing if DDFP-treatment would provide adequate oxygenation in air-breathing pigs, similarly made anemic. The treatment consisted of intravenous injection of an extremely small dose of a 2 % DDFP emulsion. DDFP has a boiling point of 29.5°C. Hence, when injected into the circulation (37°C), the emulsion particles undergo phase transition and generate microbubbles of sub-capillary diameter. The DDFP gas in the bubble is very poorly soluble and therefore "volume stabilizes" the bubbles. The bubbles take up oxygen in the lungs by diffusion and deliver it into the tissues. Because other types of bubbles are known to cause complement activation, it was hypothesized that DDFP bubbles might also do so. Therefore, experiments were conducted with DDFP emulsion and fresh human serum as a source of complement. Furthermore, tissue samples from the animals underwent histological examination to detect possible untoward effects of the treatment.

BODY

Methods

Erythrocyte replacement with volume-stabilized microbubbles

Six control pigs and 6 experimental pigs, weighing between 20 and 30 kg, were studied. The animals were anesthetized with pentobarbital (i.v.), given atropin and intubated. They were invasively instrumented for continuous recording of arterial and central venous pressures (Cobe Pressure Transducers, Denver, CO), heart rate, and O_2 and CO_2 tensions in skeletal muscle with subcutaneous electrodes (Kontron Instruments, Zürich, Switzerland) (Fig 1). All pressures were recorded on a multi-channel recorder (Gould Instruments, Lakeview, OH). Frequent sampling of blood for determination of arterial O_2 (PaO_2) and CO_2 ($PaCO_2$) tensions, acid-base status and DDFP content (treatment animals only) was made and measurements of urine production was obtained via a bladder catheter. The blood gases and pH were measured on a Ciba-Corning blood gas system (Model 278, Chiron Diagnostics Corp., Norwood, MA) and hemoglobin (HB) measured by a Gilford photometer. Using a roller pump, blood was drawn from an arterial line at a rate of 15-20 ml/min, and the same amount of 6% dextran in Ringer's solution was infused into a femoral vein. The rate of bleeding and volume replacement was guided by frequent hemoglobin (Hb) analyses so that 50% of the circulating Hb was removed in approximately 50 min.

The pigs were ventilated at a rate of 22-26 breaths/min with a tidal volume of 275-350 ml/breath, $PaCO_2$ being kept at 39-42 mm Hg. With this ventilation level PaO_2 was only approximately 80 mm Hg due to atelektases and shunting. Therefore, a small amount of oxygen (1-4%) was added to the inspired air to keep arterial oxygen tension stable between 95 and 105 mm Hg (normoxia) in the animals.

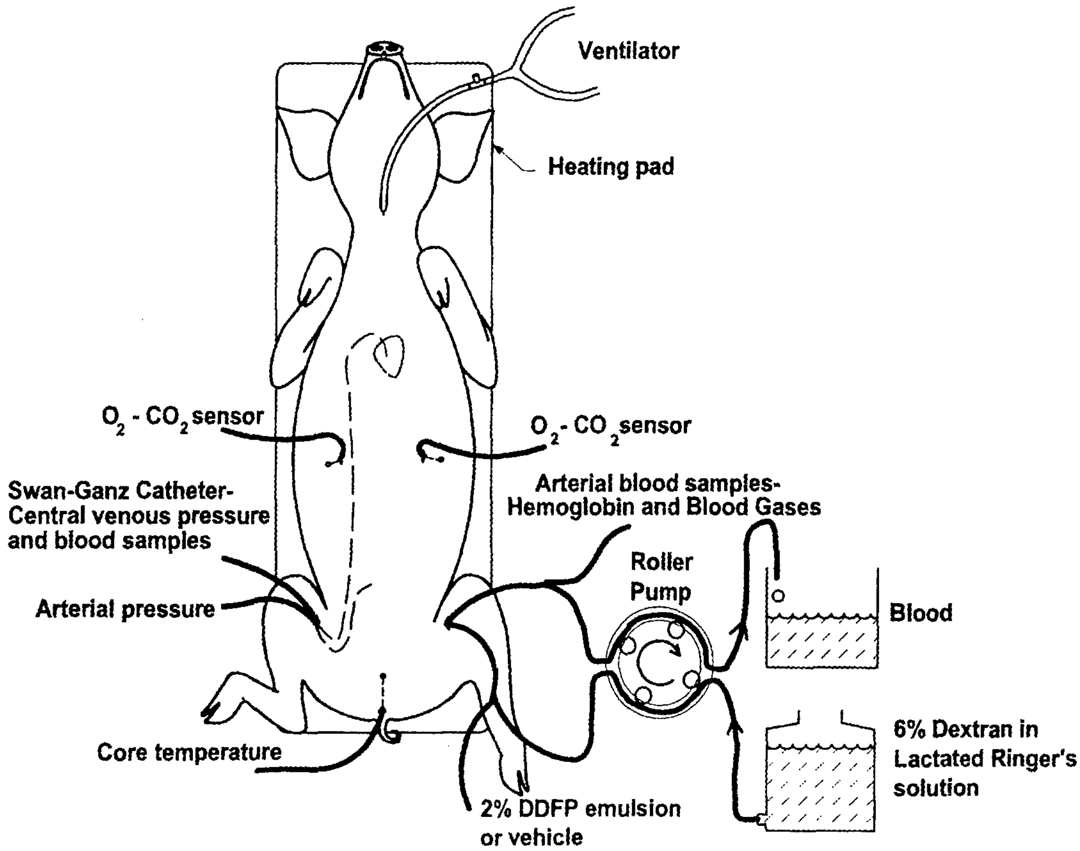

Fig 1. Schematic of preparation in anesthetized pig for erythrocyte depletion by blood withdrawal and volume replacement and for physiological monitoring while treating animal with 2% DDFP emulsion or blank (control).

The control animals were monitored until death after which tissue samples were harvested for histology.

The treatment animals underwent the same procedures as the control animals up to the point at which the circulating Hb level was 50% of the normal. At this time, intravenous infusion of a 2% DDFP in water emulsion (DDFPe, Sonus Pharmaceuticals, Inc., Bothell, WA), and 4 ml/min of Ringer's solution, for dilution of the DDFPe, was commenced at a rate of 0.2 ml/min (based on upscaling from our rat experiments (1), and continued for 120-250 min past the time period at which the control animals had succumbed to anemia. The bleeding and volume replacement, combined with DDFP emulsion administration, was continued until a considerably lower Hb level was reached than the level at which the control animals died. After this point of time, the test animals were sacrificed with an overdose of pentobarbital. In no case was there, at the time of sacrifice any indications from the physiological monitoring or otherwise to suggest that the test animal could not have continued to live.

DDFP measurements in blood

A gas chromatographic method was used to measure the amount of DDFP in blood according to a method described by Sonus Pharmaceuticals (4).

Complement system studies

Serum was obtained from normal human subjects by drawing peripheral blood, allowing it to clot at room temperature for 15 min and then, incubating this at 4° C for 2 hrs. The serum was harvested by pipets, separated into aliquots and stored at –70 °C prior to use.

The DDFP emulsion in capped vials was maintained at room temperature until use. For the experiment a one ml system was used with varying amounts of DDFP and serum incubated at 37°C for 30 min. A variety of controls were necessary including as follows: 1. Emulsion was pre-incubated at 37°C so that the bubbles dissipated in air ("debubbled") and 2. Samples containing EDTA to prevent all activation ("0 time") were tested. Following incubation of test and control tubes the mixtures were centrifuged and the supernatant reaction mixtures were harvested for analysis of C5a, C3a and C4a.

Assessment of Morphology

After sacrifice of the animals the following tissues were removed and fixed in neutral buffered formaldehyde: heart, lungs, adrenal gland, kidney, brain, cerebellum, liver, spleen, gastrointestinal tract (stomach, large and small intestine), fat, and skeletal muscle. The lungs (or a lobe of the lung) were inflated through the airway with formaldehyde. Tissues were processed by routine techniques for light microscopy. Sections (4.0 μm) were stained with hematoxylin and eosin and coded for blinded examination. Specimen were examined by light microscopy and assessed for pathological changes, including necrosis and inflammation.

Results

Hemodilution studies

The hemodilution was identical in the two groups of pigs as shown by the changes in Hb concentration against time in Fig 2. The control pigs died at a Hb level of 3.0 $\pm$ (SE) 0.1g/100 ml which was reached at 258 $\pm$ 7 min. By contrast, the test pigs survived for at least 100 min longer at a Hb level of 2.1 $\pm$ 0.1 g/100 ml. They were intentionally sacrificed while still in a viable condition. The PaO_2 and $PaCO_2$ tensions remained identical in the two groups of pigs, until the control pigs died (Figs 3-4).

The arterial pressures (Figs 5-7) were normal in the two groups of pigs until a Hb concentration of approximately 5 g/100 ml when the pressures started to decline in the control pigs and then began to fall abruptly at 3.5 g Hb/100 ml. The test pigs had a modest reduction in systolic blood pressure from 134 $\pm$ 8 mm Hg pre-hemodilution to 110 $\pm$ 4 mm Hg during the post-hemodilution period ($p< 0.01$) (Fig 5). During the same periods the diastolic pressure (Fig 6) fell from 97 $\pm$ 7 mm Hg to 55 $\pm$ 4 mm Hg ($P<0.01$) and the mean arterial pressure from 114 $\pm$ 7 mm Hg to 80 $\pm$ 5 mm Hg ($p<0.01$) (Fig 7). The heart rate shown in Fig 8 increased from 132 $\pm$ 15 beats/min to 190 $\pm$ 7 beats/min ($p<0.01$). Central venous pressure (CVP) increased similarly in both groups from 2 mm Hg up to 7 $\pm$ 2 mm Hg ($p<0.01$) for the first 2.5 hrs of hemodilution, when in the test pigs it continued to increase to 10 $\pm$ 2 mm Hg

(p<0.01). After the DDFP infusion was stopped the CVP fell to 4 ± 2 mm Hg over the next couple of hours (Fig 9).

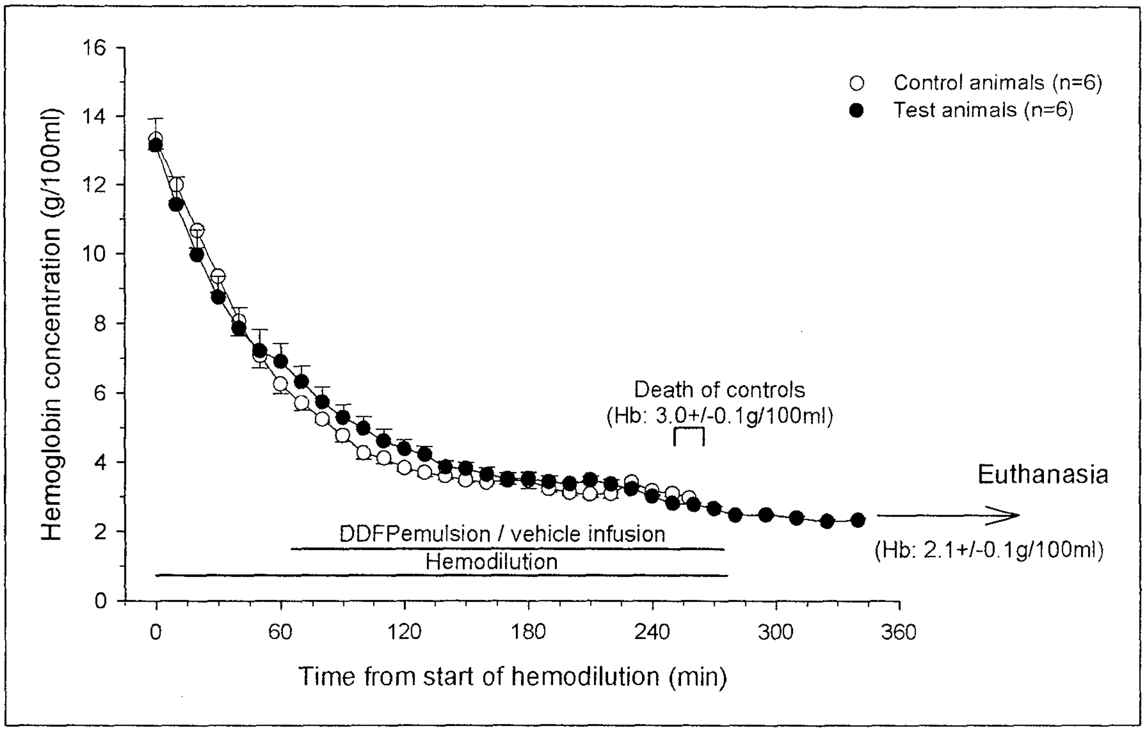

Fig 2. Erythrocyte depletion in pigs by bleeding and hemodilution: hemoglobin concentration vs. time. Data points show means and SE.

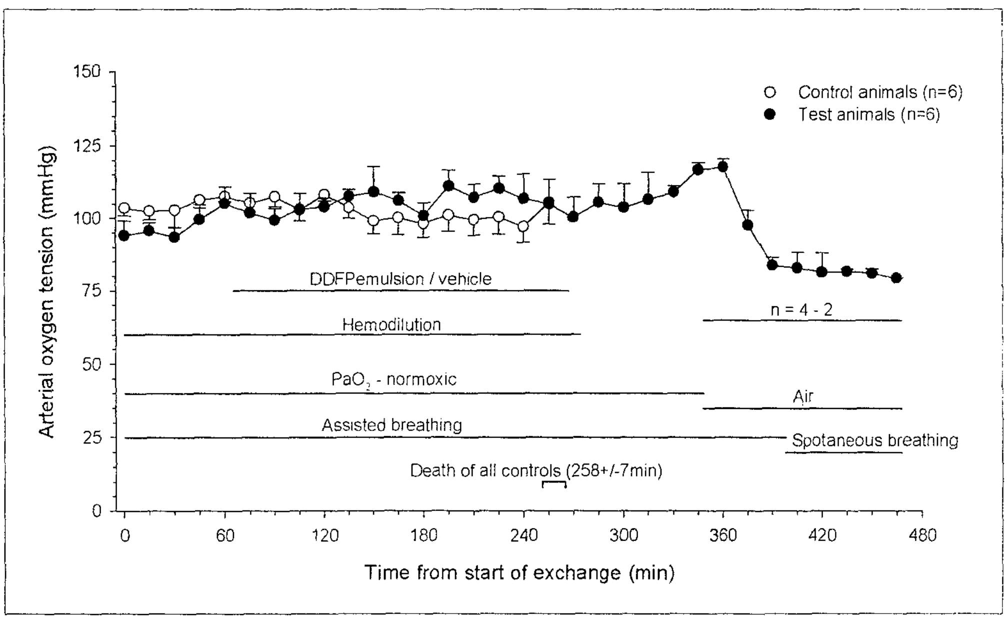

Fig 3. Arterial oxygen tensions vs. time in experiments with erythrocyte depletion; test animals received treatment with DDFP emulsion (0.7 ml/kg) and controls received preparation blank.

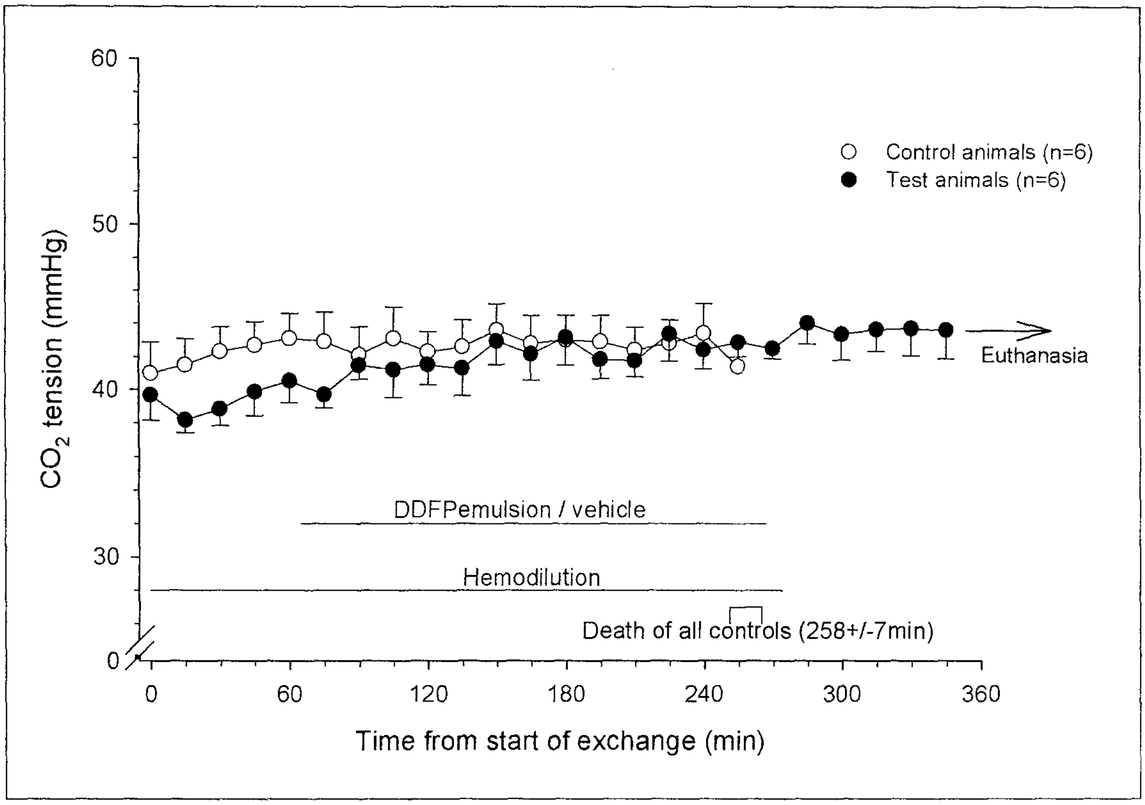

Fig 4. CO_2 tension in arterial blood vs. time

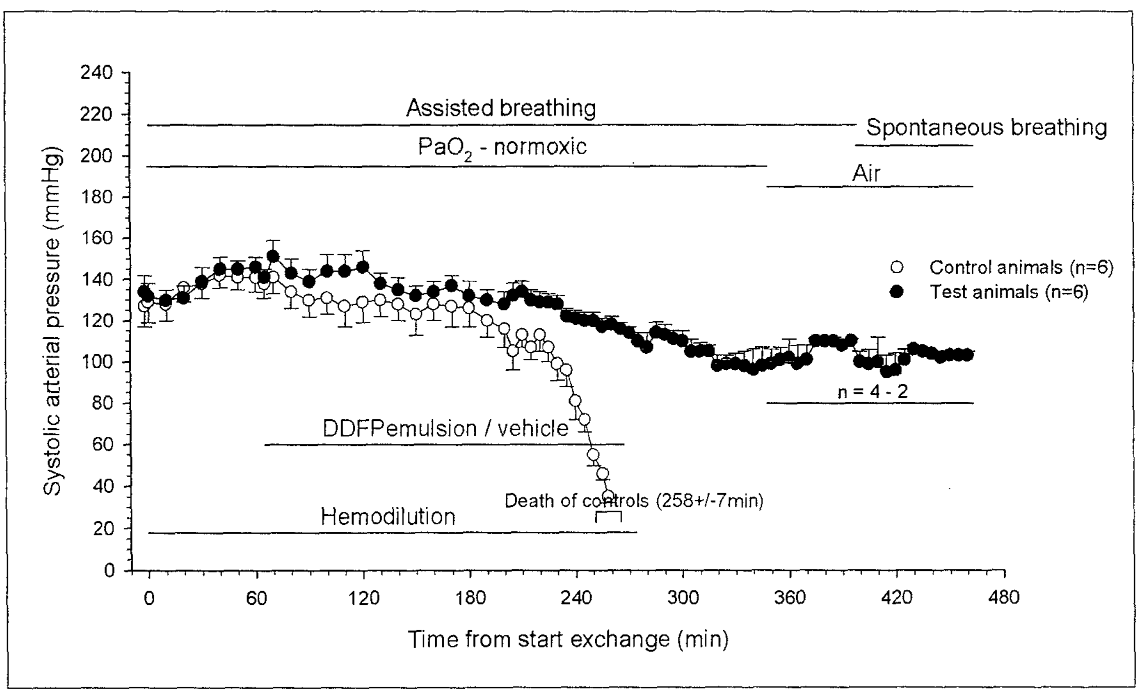

Fig 5. Systolic arterial pressure vs. time. Note that in 4 animals the slightly hyperoxic gas mixture (see text) was changed to air breathing at 350 min and artificial respiration was terminated at about 400 min. During the time period marked (n=4-2), observation time for different animals varied (see text).

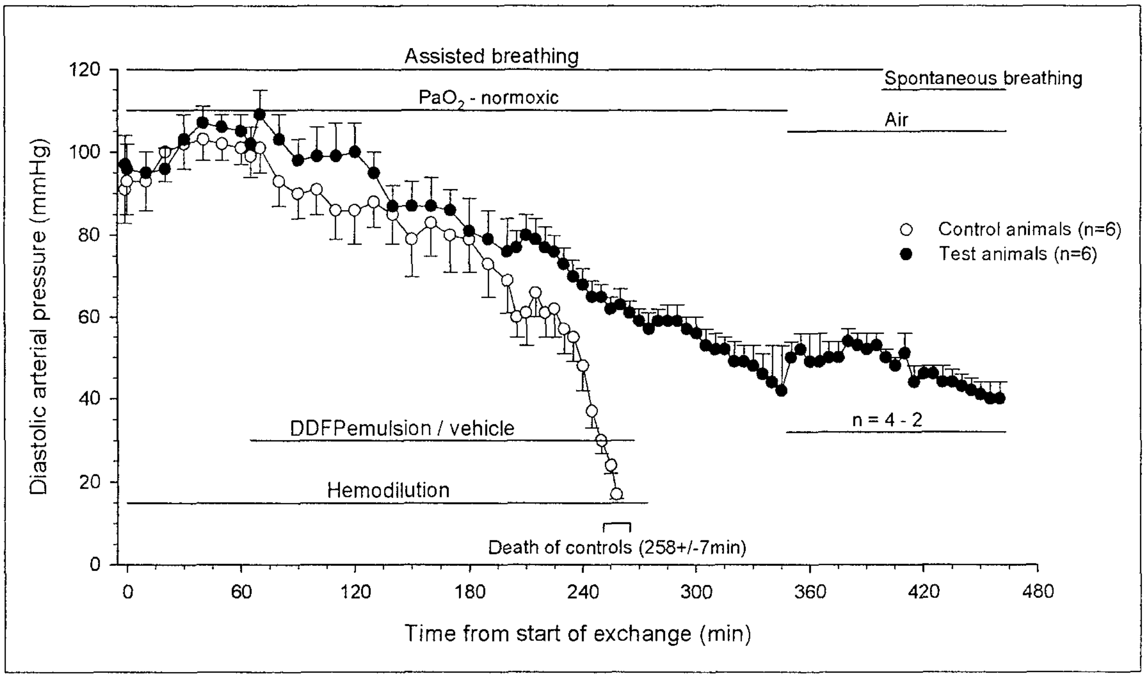

Fig 6. Diastolic arterial pressure vs. time. For additional information see legend of Fig 5.

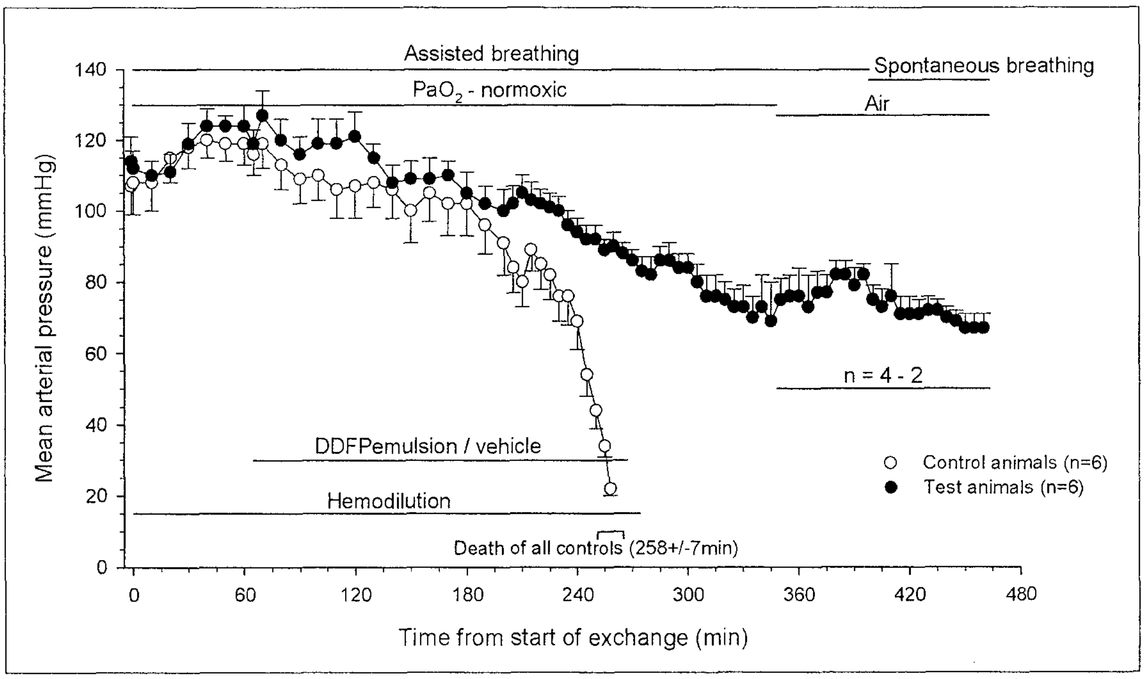

Fig 7. Mean arterial pressure vs. time. For additional information see legend of Fig 5.

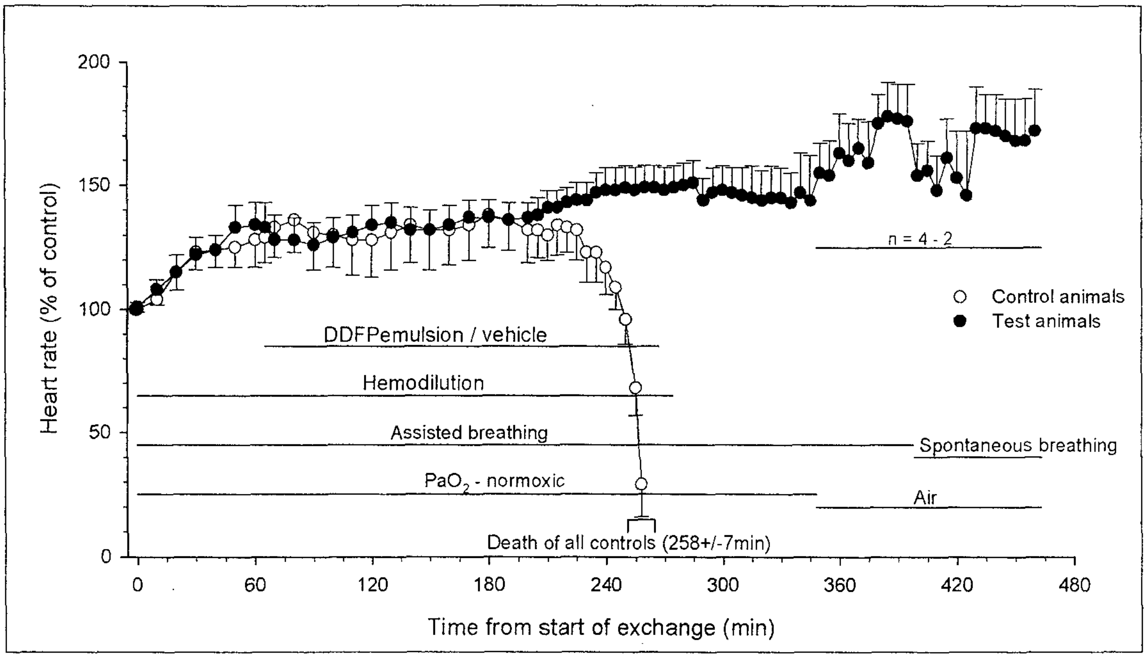

Fig 8. Heart rate in % of values at time zero vs. time. Heart rates at zero time: control animals 158+/-15 beats/min, test animals 129+/-13 beats/min. For additional information see legend of Fig 5.

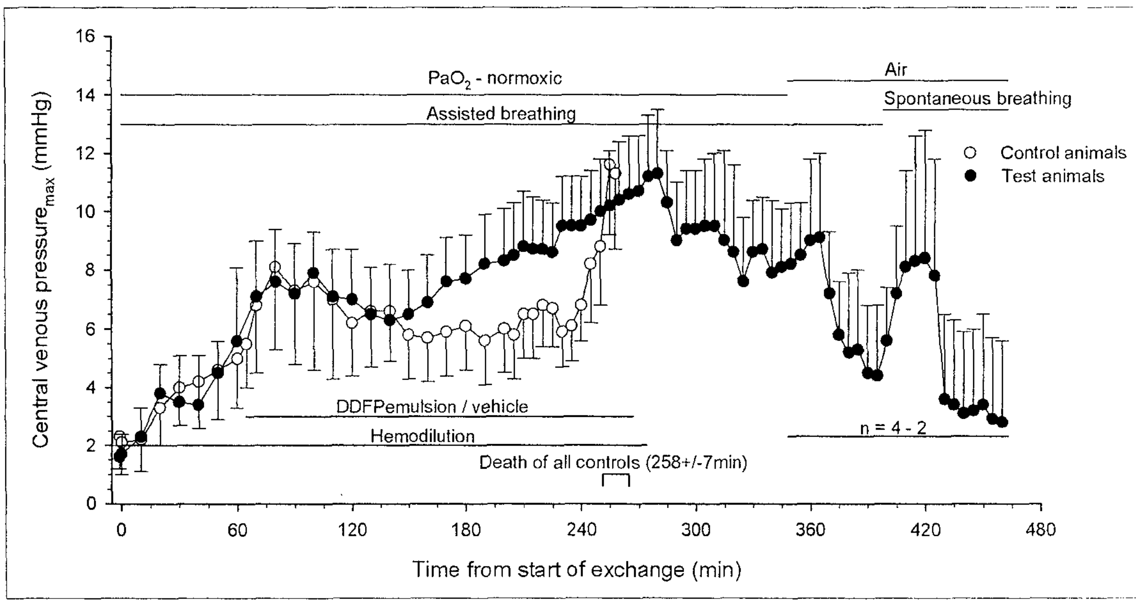

Fig 9. Central venous pressures vs. time. For additional information see legend of Fig 5.

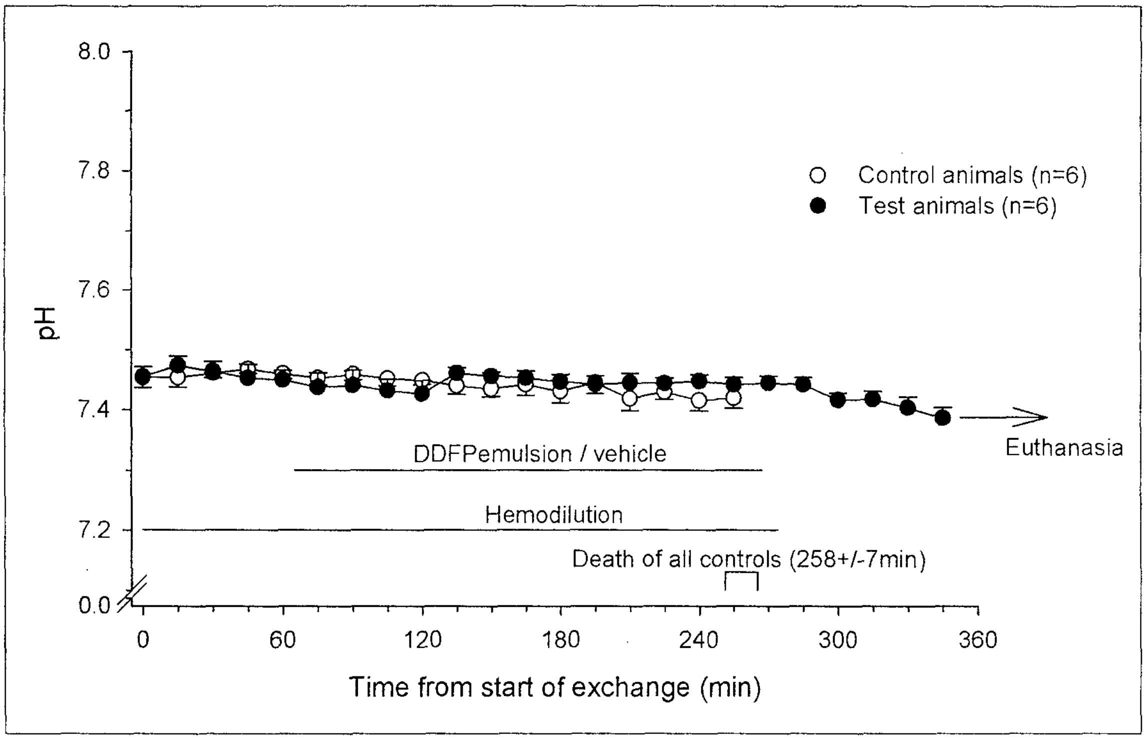

Fig 10. Arterial blood pH vs. time

The arterial pH remained in the normal range for all animals (Fig 10). The base excess values for the control animals was 5.0 ± 1 mMol/L at the beginning of the experiments and had fallen to 1.9 ± 0.7 mMol/L at the beginning of the final drop in blood pressure. By contrast, in the test animals, the base excess starting at 5.4 ± 0.6 mMol/L had not changed (5.0 ± 0.1 m Mol/L) at the end of the protocol-prescribed observation period. Since the central venous O_2 ($P\bar{v}O_2$) tension reflects the average of all oxygen tensions in all tissues, a higher O_2 tension would indicate a better oxygenation of the tissues. In Fig 11 the $P\bar{v}O_2$ in the two animal groups is shown in per cent of the values before start of the experiments (53 ± 3 and 50 ± 2 mm Hg in control and test pigs, respectively). This figure shows a significantly higher O_2 tension for the whole time period the test animals were given DDFP and they remained at $P\bar{v}O_2$ of approximately 30 mm Hg from 2 hrs into the hemodilution process and until euthanasia. Skeletal muscle O_2 remained normal or at viable levels for the whole experimental period in test pigs (50 to 30 mm Hg), but declined in the control pigs (Fig 12). On the other hand, after 3.5 hrs at minimal Hb concentration the tissue CO2 was higher (at about 60 mm Hg) than the pre-exposure control in test pigs (about 53 mm Hg) (Fig 13).

The protocol required that test animals should survive for 1 h longer than the controls while maintaining a $P\bar{v}O_2$ of at least 30 mm Hg. This requirement was met with a Hb concentration that was actually only 70% of that at which the controls died. Indeed, in four pigs the $P\bar{v}O_2$ did not decrease after change to air breathing and spontaneous ventilation.

<u>Measurements of DDFP concentration in blood.</u>

The concentration of DDFP was measured repeatedly in arterial blood during DDFP infusion and for up to 3 hrs after the infusion was terminated (Fig 14). The plasma concentration of DDFP was 0.3 ± 0.05 μl/ml. Within 30 min after the infusion was stopped, the concentration fell to 0.08 ± 0.02 μl/ml and remained fairly stable for the next 2-3 hours.

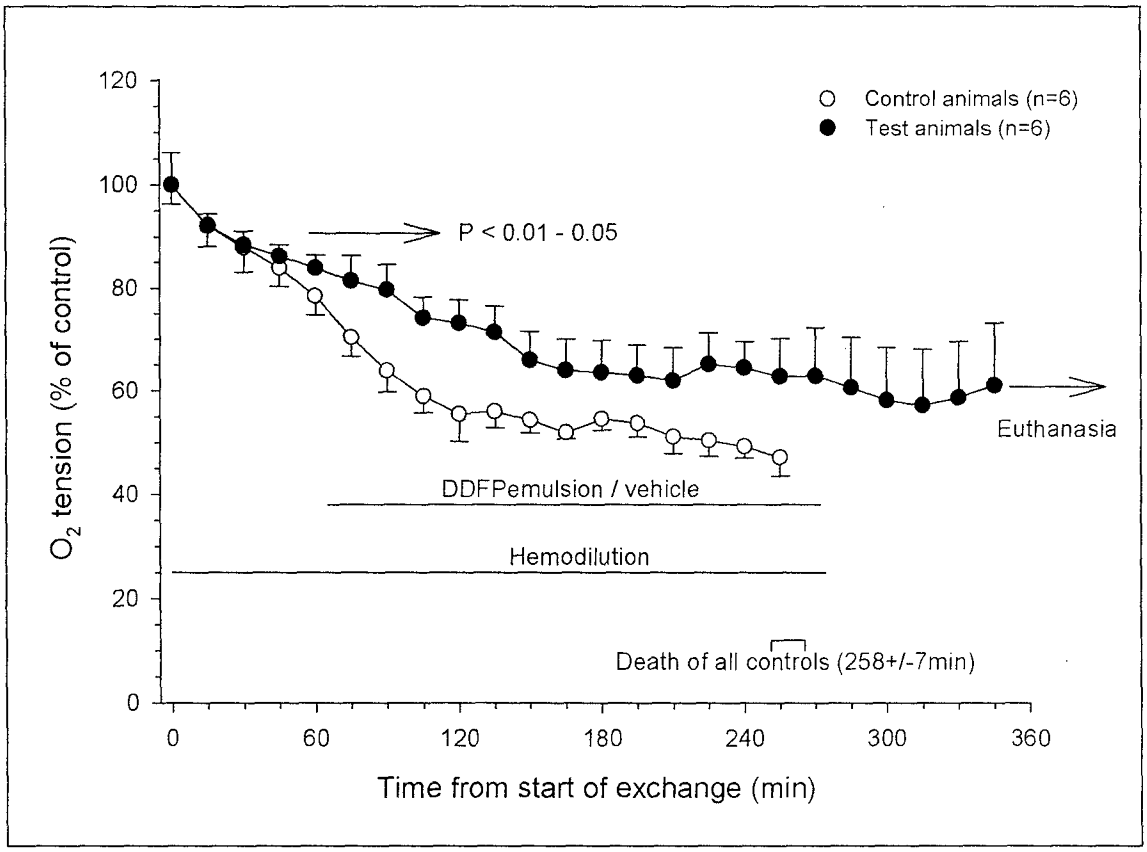

Fig 11. Oxygen tension in mixed venous blood vs. time. Data points expressed as percent of control values measured in the two groups at time zero (for absolute values see text).

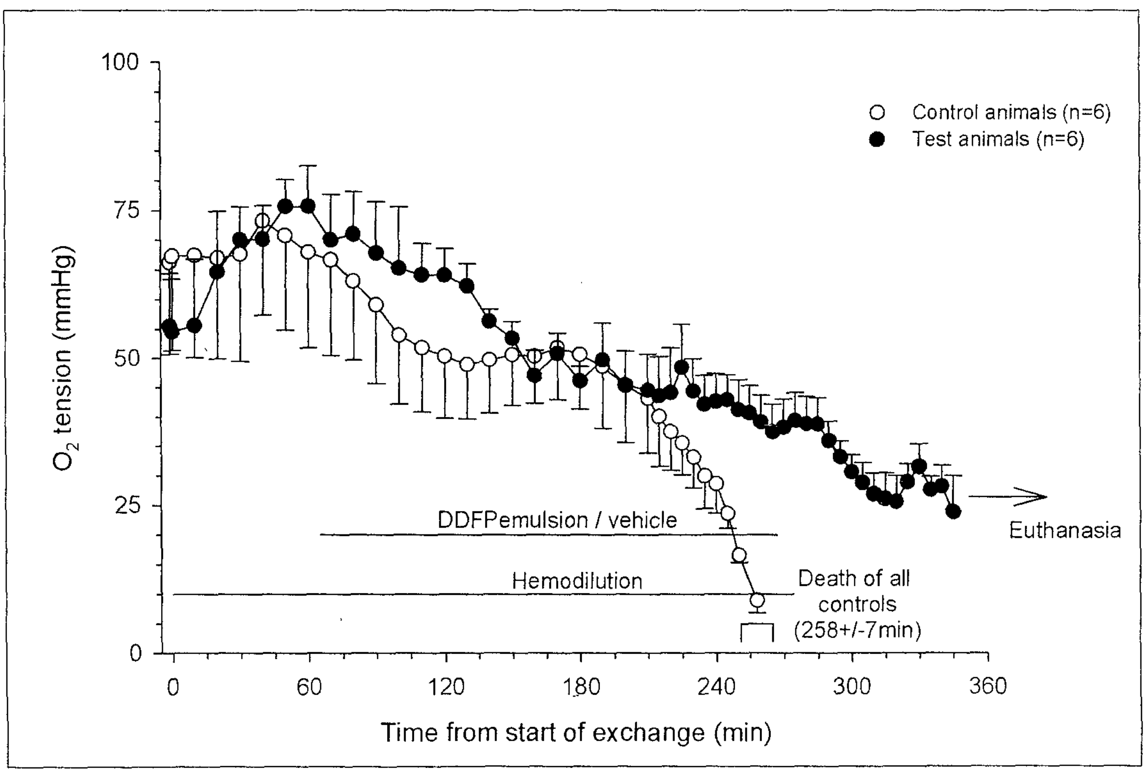

Fig 12. Abdominal muscle oxygen tension vs. time

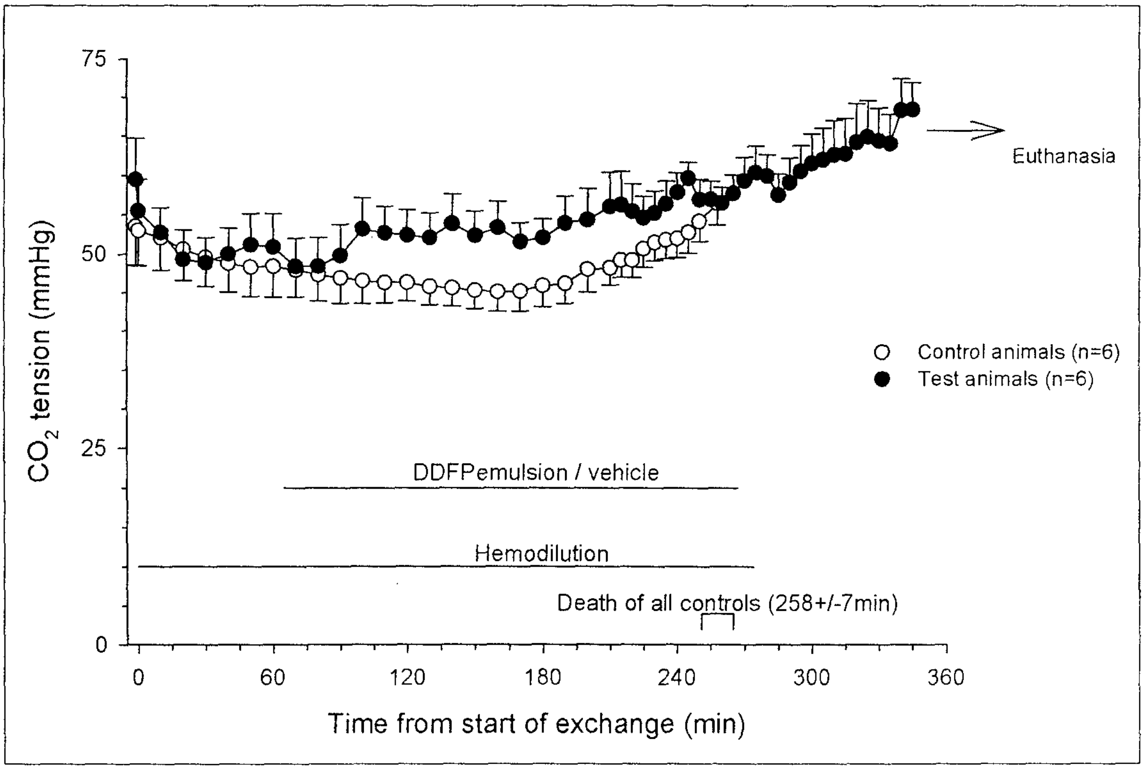

Fig 13. Abdominal muscle CO_2 tension vs. time

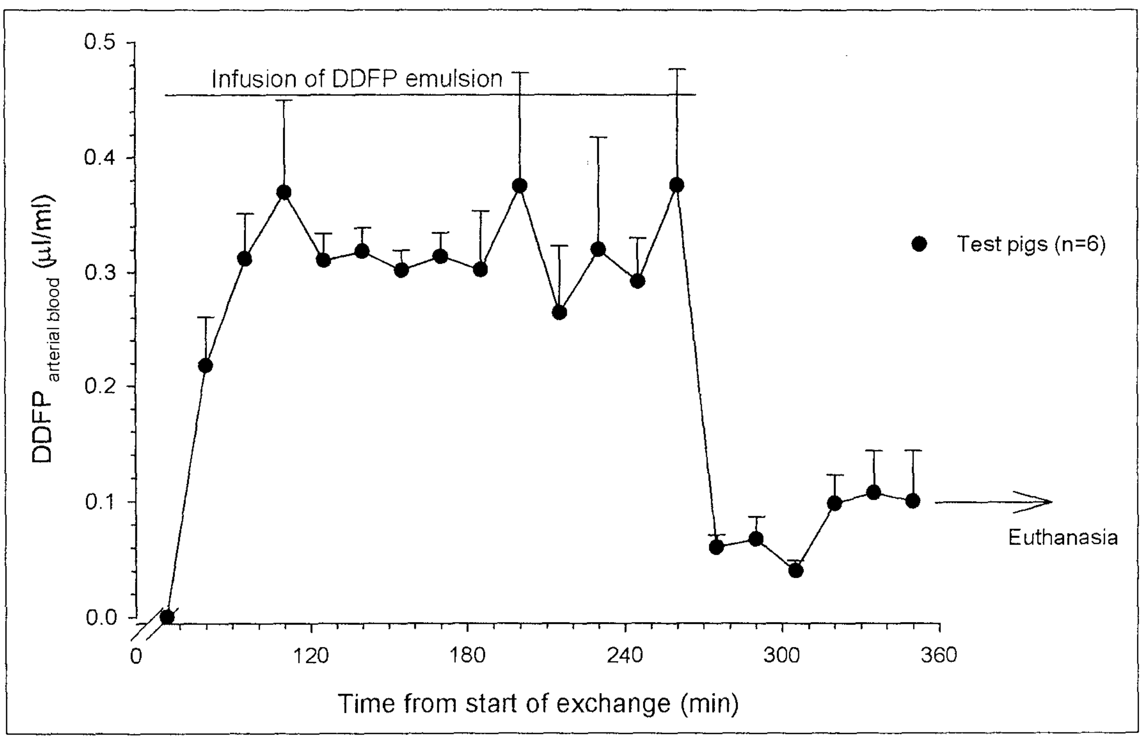

Fig 14. DDFP concentration in arterial blood vs. time in test animals

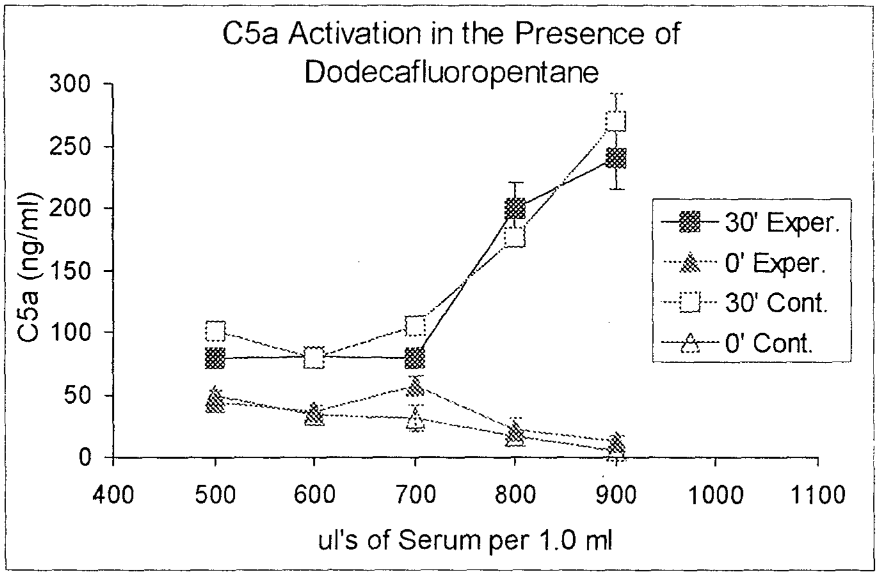

Fig 15. Activation of complement protein fragment C5A in human serum incubated with DDFP emulsion. Horizontal axis shows serum concentration in 1.0 ml of serum-DDFPe mixture. Four conditions were tested: no activation controls (EDTA), (1) one with degassed DDFPe blank (squares) and (2) one with DDFPe (circles); (3) experiment with degassed DDFPe incubated for 30 min (stars) and (4) experiment with fresh DDFPe incubated for 30 min (triangles).

Complement activation

Measurement of complement protein fragments C4a, C3a and C5a after incubation with DDFP bubbles showed no additional complement activation over controls (Fig 15). The results of the C5a testing are shown in Fig 15. At higher serum concentrations C5a was detected in supernatants of both test and control mixtures showing nonspecific complement activation at very high serum concentration, but at no serum concentrations was a significant difference seen between the amount of C5a detected with DDFP bubbles as compared to the debubbled material. Testing of C3a showed similar results with increasing nonspecific activation at high serum concentrations. As with C5a testing no difference was seen between activation with and without bubbles. Analysis of C4a in the supernatant showed no significant activation in experiments or controls. These results are shown in Fig 16 which depicts an experiment in which 700 μl of serum and 300 μl of DDFP were used. Thus, these *in vitro* experiments show no evidence of DDFP-bubble induced ability to activate complement in the presence of fresh human serum.

Discussion

The central hypothesis of this study was that intravenous infusion of a 2% DDFP emulsion can sustain life for an hour in anesthetized air breathing pigs that have been erythrocyte depleted to a hemoglobin level which is fatal in untreated animals. This hypothesis was supported. Indeed, several animals were observed for up to 4 hrs without showing signs of significant deterioration. Some animals had low PaO_2 values during part of the experiment. This was probably due to ventilation/perfusion abnormalities caused by the anesthesia and the fact that the animal was lying on its back. This interpretation is supported by the fact that a small oxygen admixture (1-4%) to the breathing air secured arterial normoxia (Fig 3).

Moreover, 4 out of 6 treated animals could be sustained on air alone for one to three hours at the end of the experiments.

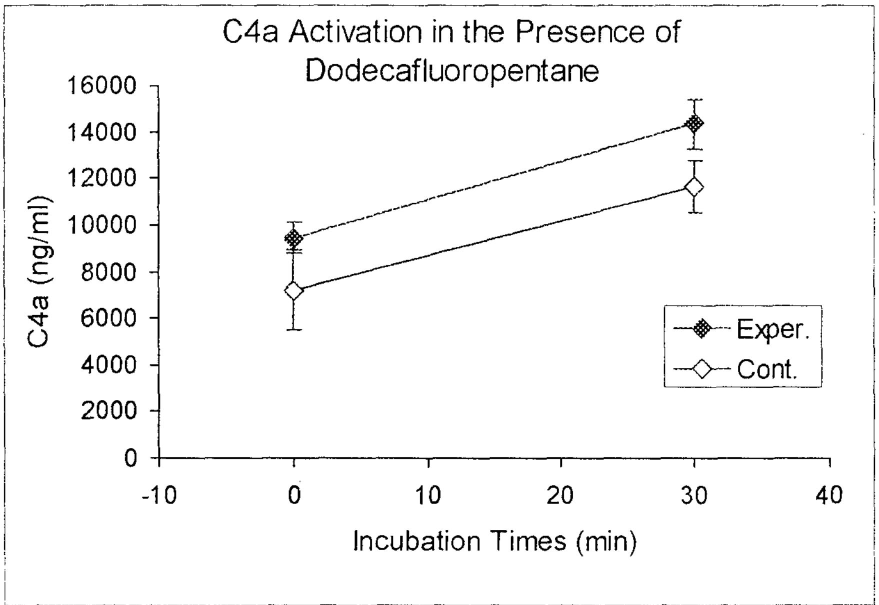

Fig 16. Activation of complement protein fragment C4a in human serum incubated with DDFPe vs. incubation time. The control experiments were performed with degassed DDFPe (squares) and the experiments with fresh DDFPe (triangles).

Critical to the success of this treatment was that it supported adequate tissue oxygenation which is indicated by the $P\bar{v}O_2$ which remained above 30 mm Hg in the test animals (cf Fig 11). Moreover, arterial pH remained stable at 7.45 (Fig 10) as did $PaCO_2$ at 40-45 mm Hg suggesting an absence of significant metabolic acidosis, a notion further supported by the base excess being normal and stable in the test animals while it fell markedly in the controls.

The CO_2 elimination was sufficient to secure normal $PaCO_2$ (Fig 4) in the test animals throughout the protocol-prescribed observation period and also well into the final drop in blood pressure in the controls. This probably reflects that a dwindling CO_2 delivery to the lung in the controls was adequately handled by the fixed ventilation.

In the test animals, the muscle PCO_2 (Fig 13) increased slightly above the initial value during the final observation period. This is in keeping with the theory for gas transport in the intravascular bubbles (2). When the bubbles arrive in the systemic capillaries, they give up oxygen and undergo a volume reduction which leads to increases in CO_2 concentration and PCO_2 causing a corresponding increase in tissue PCO_2.

As for the circulatory parameters, the systolic arterial blood pressure in the test animals underwent a modest decrease (Fig 5). The more marked reduction in mean arterial pressure (Fig 7) was reflective primarily of a large drop in diastolic pressure (Fig 6). The latter may have been caused mostly by the lower viscosity of the nearly cell-free circulating liquid. The changes in pressure caused an increase in pulse pressure from less than 40 mm Hg at the beginning of the experiments to more than 60 mm Hg at the end of the protocol-prescribed hour of observation. These changes are likely to have caused the

pressor response in the heart rate which increased by 50% (Fig 8). The increase in central venous pressure (Fig 9) in the test animals from the beginning of the experiment to the end of the DDFP infusion period was probably due to the volume load introduced with the treatment. Shortly after the end of the DDFP infusion, the CVP began to fall and was near normal during the extended observation period. For the purpose of exemplification, one may consider the experiment in a 30 kg pig: over the 195 min long period of i.v. DDFP administration, addition of 4 ml of Ringer's solution will introduce 780 ml of fluid, 20% of which i.e. 156 ml, can be estimated to remain in the circulation. Additionally, the DDFP emulsion infused provides a total dose of 0.42 ml of DDFP which, on phase transition, expands about 150 times to 63 ml, and this volume is further increased by 5 times when the bubbles take up oxygen, nitrogen and carbon dioxide so as to occupy a volume of 315 ml (cf 3). Thus, the total volume expansion of the fluid volume can be expected to exceed the normal blood volume by about 20%. This might well explain the increase in CVP in the test animals during the treatment phase as well as the subsequent drop in CVP thereafter which is likely to reflect volume loss via the urine and gradual reduction in free gas volume primarily due to DDFP elimination which is known to occur via the lungs (5).

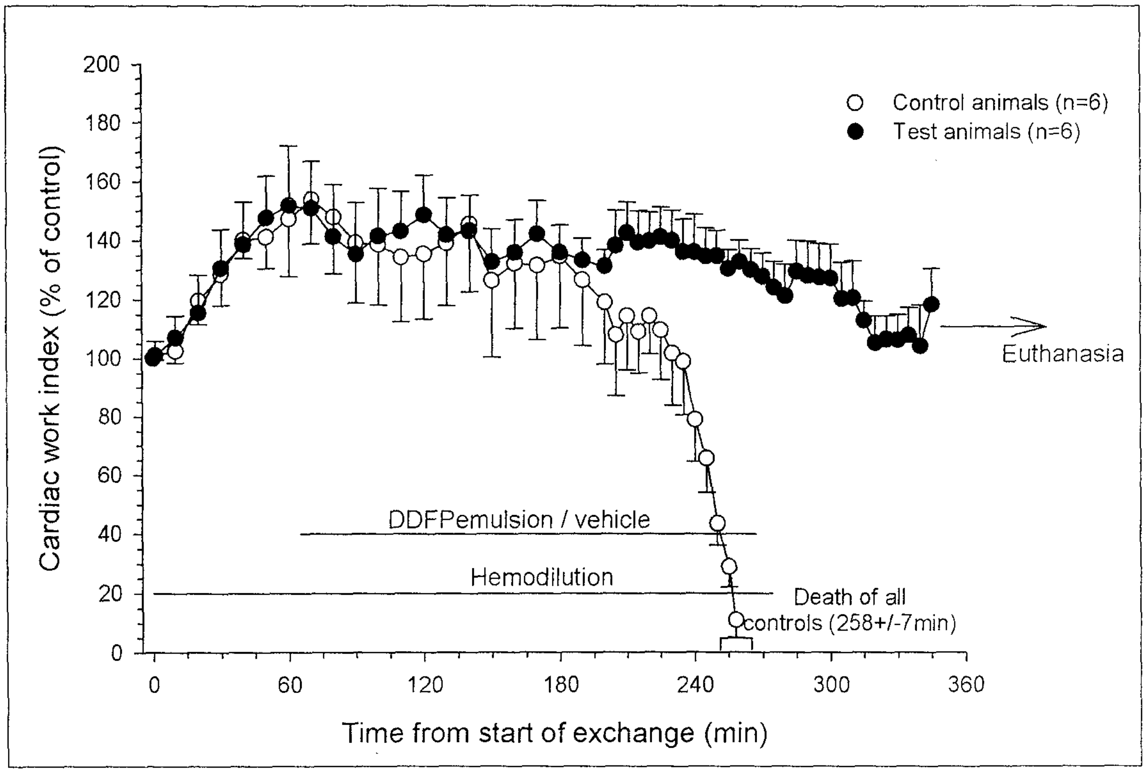

Fig 17. Cardiac work index (heart rate • systolic arterial pressure) vs. time

The circulatory parameters discussed above indicate that the test animals remained in acceptable physiological condition. This is further supported by the calculation of the cardiac work index illustrated in Fig 17 which shows that through the first hour of hemodilution the index increased by 50% in both groups of animals and then remained at a plateau during the ensuing 3 hrs after which there was a precipitous drop until death in the control animals. By contrast, the index was gradually reduced and leveled off at the control value during the final hour of observation in the six test animals. Thus, the cardiac performance was satisfactory in these animals.

Judging from the therapeutic effects being sustained for up to 4 hrs after termination of the DDFP infusion, an adequate amount of bubbles must have remained in the circulation for at least that length of time. Although the amount of DDFP gas in the blood fell, after the infusion, to only 25% of the level during infusion (Fig 14), the remaining gas in combination with nitrogen, which is poorly soluble, appears to have provided sufficient longevity of the bubbles.

Complement activation was not seen when human serum was incubated with DDFPe even in very high concentrations (Figs 15 and 16). These results differ from the complement activation seen *in vitro* when nitrogen bubbles are present (7). The reason for the DDFP bubbles being more neutral relative to the complement system than nitrogen bubbles is not clear but the possibility that the surface characteristics of the two types of bubbles differ should be considered.

Blinded histological examination (light microscopy) was performed on tissue samples from heart, lung, spleen, liver, stomach, duodenum, colon, kidneys, suprarenal glands, brain, cerebellum, spinal cord, subcutaneous and abdominal fat, eye, and skeletal muscle. No abnormalities were found in either controls or test animals.

KEY RESEARCH ACCOMPLISHMENTS

- Intravascular microbubbles generated by injection of a 2% dodecafluoropentane (DDFP) emulsion can replace the erythrocytes for oxygen transport in air breathing anesthetized pigs.

- Only 0.014 ml DDFP/kg bodyweight is required for life sustaining oxygenation for several hours in the erythrocyte depleted pig.

- No ill effects were observed in circulatory parameters, complement activation (human serum *in vitro*) or multiorgan histology in the animals.

REPORTABLE OUTCOMES

This work has been described in two abstracts and presented at three scientific meetings:

1. I. Tyssebotn, G. W. Bergoe, and C. E. G. Lundgren. Volume-stabilized intravascular microbubbles provide vital oxygenation in hemoglobin depleted pigs during "air breathing". Undersea & Hyperbaric Medicine, Vol. 28, Abstract E30, 2001, Supplement. Presented at Undersea and Hyperbaric Medical Society Annual Scientific Meeting, 14-16 June, 2001, San Antonio, Texas.

2. Ingvald Tyssebotn, Guri Bergoe, and Claes Lundgren. Extremely low doses of dodecafluoropentane emulsion sustain life and function in erythrocyte depleted animals. Program of 29th ISOTT (International Society on Oxygen Transport to Tissue) Annual Meeting, Philadelphia, PA, USA; August 11-15, 2001, p. 121.

3. C. Lundgren, G. Bergoe, I. Tyssebotn. Extremely low doses of dodecafluoropentane emulsion sustain life and function in erythrocyte depleted animals. Program of

ATACCC 2001 (Advanced Technology Applications for Combat Casualty Care), Fort Walton Beach, FL, September 9-14, 2001, p. 16.

These abstracts are attached under "Appendices".

A grant proposal based on the present work and entitled "Treatment of hemorrhagic shock, in a porcine model, with oxygen carrying intravascular microbubbles" has been submitted to the US Army Medical Research Acquisition Activity. The proposal has been approved.

CONCLUSIONS

Microbubbles generated in the circulation by intravenous infusion of a very small dose of a 2% dodecafluoropentane emulsion (DDFPe) can provide life sustaining oxygenation in air breathing pigs in which bleeding and volume replacement with a plasma expander have reduced the hemoglobin to a level that is lethal in control animals.

Adequate tissue oxygenation was indicated by normal mixed venous oxygen tensions as well as physiological muscle PO_2 levels in treated animals. Further indication of an adequate circulation was a normal cardiac work index.

Systolic and mean arterial pressures remained well above shock levels in test animals while diastolic pressure leveled off at 40-50 mm Hg, probably reflecting the low viscosity of the circulating fluid. Central venous pressure in the test animals underwent a sustained moderate increase during the DDFPe infusion and then fell back to control levels. This was probably due to a passing volume "overload" caused by the volume expansion of the circulating fluid caused by the DDFP gas formation. Such a volume expansion may be beneficial in combat casualty care since it may reduce the need for liquid plasma expanders.

The DDFPe was tested for *in vitro* activation of the complement system in human serum. No activation was observed. Tissue samples from 15 different organs harvested after death showed no abnormalities on light microscopy. These observations of a lack of deleterious effects suggest that the treatment may be safe as used, although additional toxicology studies are called for especially with consideration of repeated (long term) treatment.

The findings in this study suggest that intravenous administration of small doses of DDFPe might enhance the prognosis of patients who suffer life threatening hypoxia due to erythrocyte loss.

The therapeutic potential of treatment with DDFPe, in combination with minimal volume replacement should be tested in a "combat casualty type" circulatory shock model.

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APPENDICES

1. Tyssebotn I., Bergoe G.W. and Lundgren C. Volume-stabilized intravascular microbubbles provide vital oxygenation in hemoglobin depleted pigs during "air breathing". Programs and Abstracts of Undersea and Hyperbaric Medical Society Annual Scientific Meeting, San Antonio, Texas, June 14-16, 2001, Abstract 30.

2. Tyssebotn I., Bergoe G.W. and Lundgren, C. Extremely low doses of dodecafluoropentane emulsion sustain life and function in erythrocyte depleted animals. International Society on Oxygen Transport to Tissue 29th Annual Meeting, Philadelphia, PA, August 11-15, 2001.

3. Lundgren C., Bergoe G. and Tyssebotn I. Extremely low doses of dodecafluoropentane emulsion sustain life and function in erythrocyte depleted animals. Advanced Technology Applications for Combat Casualty Care, Fort Walton Beach, FL, September 9-14, 2001, Abstract 0.

(The contents of abstracts Nos. 2 and 3 are the same.)

PROGRAM AND ABSTRACTS

PRE-COURSES 13 JUNE 2001:

1. "MECHANISMS OF DECOMPRESSION ILLNESS III"
2. BAROMEDICAL NURSES ASSOCIATION
3. "PATIENT SAFETY" WORKSHOP

UNDERSEA AND HYPERBARIC MEDICAL SOCIETY ANNUAL SCIENTIFIC MEETING
14-16 June 2001

Adam's Mark Hotel
SAN ANTONIO, TEXAS, USA

E 29 **A DESCRIPTIVE ANALYSIS OF MIDDLE EAR BAROTRAUMA IN PATIENTS UNDERGOING HYPERBARIC OXYGEN THERAPY** SE Hunter, JJ Freiberger, GDL Dear, BW Stolp, RE Moon, Department of Otolaryngology Head and Neck Surgery, Department of Anesthesia and the Duke University Center for Hyperbaric Medicine and Environmental Physiology, Durham, NC 27710

BACKGROUND: Otic barotrauma (OB) is common in patients undergoing hyperbaric oxygen therapy (HBO). While there is anecdotal evidence that head and neck disease, advanced age and prior radiation are risk factors for OB, there are no reports in which risk factors have been systematically analyzed.

METHODS: Data on a consecutive series of patients treated with HBO in 1999 N=166 were reviewed for risk factors for OB. Factors analyzed were: age, gender, neurological impairment, indication for HBO, prior radiation, head and neck disease, history of ear disease, and current tobacco and/or alcohol use. Patients receiving pre-treatment tubes or myringotomy (N=23) were excluded from the analysis.

RESULTS: Twelve of 128 cases had OB. No OB was reported in patients treated for decompression illness, crush injury or acute ischemia. The diagnoses and risk factors for OB are illustrated in the table below.

DIAGNOSIS	N	%OB		VARIABLE	ODDS RATIO	95% CI
Osteoradionecrosis	4	33.3%		Age>55	5.70	1.22, 26.73
Soft tissue radionecrosis	4	33.3%		Male gender	0.13	0.03, 0.58
Necrotizing infection	2	16.7%		Head/Neck disease	4.22	0.96, 18.53*
Skin graft prep	1	8.35%		Current EtOH use	2.15	0.91, 5.08*
Osteomyelitis	1	8.35%				* NS

DISCUSSION: Based upon analysis of 1999 data, we conclude that age greater than 55 and female gender are predictors of OB in patients treated with HBO. Alcohol use, neurologic impairment, history of ear disease, current tobacco use, prior radiation and the presence of head and neck disease were not significant in this analysis.

E 30 **VOLUME-STABILIZED INTRAVASCULAR MICROBUBBLES PROVIDE VITAL OXYGENATION IN HEMOGLOBIN DEPLETED PIGS DURING "AIR BREATHING"** I Tyssebotn, GW Bergoe, CEG Lundgren Center for Research and Education in Special Environments and Department of Physiology and Biophysics, School of Medicine and Biomedical Sciences, State University of New York at Buffalo, NY 14214

Intravascular microbubbles generated by i.v. infusion of a dodecafluoropentane emulsion (DDFPe) can transport physiologically significant amounts of oxygen. Thus, rats rendered anemic by bleeding and plasma replacement survived when treated with DDFPe and oxygen, while oxygen breathing controls died (1). The present study explores the question whether DDFPe treatment can similarly sustain life during air breathing in anesthetized pigs. Pigs were bled while given volume replacement with 6% dextran in lactated Ringer's solution. In all animals, measurement of PaO_2 was performed every 15 min. If needed, artificial ventilation and/or oxygen admixture (never > than 5%) to the inspired air was given to maintain PaO_2 in the normoxic range of 90-110 mm Hg. A group of control animals (n=6) received emulsion blank in addition to the plasma expander. They died at a mean hemoglobin level of 2.75 g/100 ml after a marked drop in systolic blood pressure which began when the hemoglobin concentration was 5 g/100 ml. The experimental animals received 0.7 ml DDFPe/kg body weight in an i.v. infusion lasting for ~190 min of the 260 min long exsanguination period. These animals survived for more than one hour at hemoglobin levels averaging 2.04 g/100 ml. They retained a normal blood pressure and $PaCO_2$ throughout the experiments.

We conclude that perfluorocarbon stabilized microbubbles hold promise as an erythrocyte substitute in situations, such as combat casualty care, even when oxygen is not readily available.

1. Tyssebotn I, Bergoe G, Goldinger J, and Lundgren C. Dodecafluoropentane (DDFP) stabilized microbubbles support life without blood in awake rats. Undersea Hyperbaric Med 26: abstract 97, 1999.

Acknowledgment: This study was supported by US Army Medical Research Acquisition Activity Grant Volume-Stabilized Intravascular Microbubbles for Circulatory Transport of Oxygen and Carbon Dioxide: A Field-Usable Concept (ID: DAMD170010514) and by Sonus Pharmaceuticals, Inc.

E 31 **VENOUS GAS EMBOLISM (VGE) CAUSED BY INFUSION IMPAIR ENDOTHELIAL FUNCTION WITHOUT MECHANICAL DAMAGE** V Nossum, A Hjelde, AO Brubakk Department of Physiology and Biomedical Engineering, Norwegian University of Science and Technology, N-7489 Trondheim, Norway.

Earlier studies have shown that gas bubbles from decompression and gas embolization lead to endothelial dysfunction and mechanical injury in pigs, rabbits and lambs. 0.01ml air per minute per kg was infused through a catheter into the

29th ISOTT annual meeting
Philadelphia, PA, USA

August 11 - 15, 2001

Extremely low doses of dodecafluoropentane emulsion sustain life and function in erythrocyte depleted animals.

Ingvald Tyssebotn , Guri Bergoe and Claes Lundgren
Center for Research and Education in Special environments and Department of Physiology and Biophysics, School of Medicine and Biomedical Sciences, State University of New York at Buffalo, NY 14214, Fax: (716) 829-2384,Email: ityssebo@acsu.buffalo.edu

It has been proposed that microbubbles derived from an emulsion of dodecafluoropentane (DDFP) administered intravenously can transport significant amounts of oxygen from the lungs to the tissues (Burkard and Van Liew, 1994). In several series of experiments in rats and pigs, we have recently tested the practicality of this mode of gas transportation and also whether it applies to inert gas.

In anesthetized rats breathing 70-100% oxygen and anesthetized pigs breathing air (oxygen enriched to arterial normoxia), blood was iso-volumetrically exchanged with an iso-osmotic plasma expander. Half of the animals were given a 2% DDFP emulsion, 0.7ml/kg i.e.14 µl i.v. DDFP/kg, and half of the animals received vehicle. The control rats died at 2.7 g hemoglobin (Hb)/100 ml and the control pigs at 3.0 g Hb/100 ml, while all test animals receiving DDFP survived at Hb levels of 1.4 (rats) and 2.0 g/100 ml (pigs) until terminated after having lived 2-4 hours longer than the control animals. One series of rats that was allowed to wake up from anesthesia exhibited normal behavior, walking, eating drinking and grooming. All test animals had normal blood pressure. After retransfusion of shed blood, the rats were kept for 21 days, after which they were sacrificed and multi-organ histology was performed. No changes were found in any specimen.

To demonstrate the general applicability of gas transport by intravascular microbubbles, we tested the rate of whole body nitrogen washout in anesthetized, spontaneously breathing pigs treated with DDFP emulsion. The treatment pigs received DDFP emulsion, 0.1ml/kg i.v. and controls were given vehicle during the first 30 min of the 120 min long oxygen breathing session. The DDFP treated pigs eliminated the same amount of nitrogen in 68 min as the control animals did in 120 min with identical cardiac output, i.e. the washout time was reduced by 43%.

Conclusion: These experiments demonstrate that both oxygen and nitrogen can be transported effectively by intravascular volume-stabilized DDFP microbubbles. If the results apply to humans, one can predict that the oxygen consumption of an oxygen breathing resting 75 kg person can be provided for by a total dose of 1.1 ml of dodecafluoropentane injected intravenously in form of an emulsion.

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Burkard, Mark E. and Van Liew, Hugh D. Oxygen transport to tissue by persistent bubbles: theory and simulations. *J. Appl. Physiol.* 77(6):2874-2878, 1994.

Acknowledgments
This study was supported by US Army Medical Research Acquisition Activity Grant "Volume-Stabilized Intravascular Microbubbles for Circulatory Transport of Oxygen and Carbon Dioxide: A Field-Usable Concept" (ID: DAMD170010514) and by Sonus Pharmaceuticals, Inc.

Combat Casualty Care Research

Advanced Technology Applications for Combat Casualty Care

ATACCC 2001

September 9 – 14, 2001
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Speaker Abstracts

-0-

EXTREMELY LOW DOSES OF DODECAFLUOROPENTANE EMULSION SUSTAIN LIFE AND FUNCTION IN ERYTHROCYTE DEPLETED ANIMALS.

C. Lundgren, G. Bergoe, I. Tyssebotn

It has been proposed that microbubbles derived from an emulsion of dodecafluoropentane (DDFP) administered intravenously can transport significant amounts of oxygen from the lungs to the tissues (Burkard and Van Liew, 1994). In several series of experiments in rats and pigs, we have recently tested the practicality of this mode of gas transportation and also whether it applies to inert gas. In anesthetized rats breathing 70-100% oxygen and anesthetized pigs breathing air (slightly enriched with oxygen to achieve arterial normoxia), blood was iso-volumetrically exchanged with an iso-osmotic plasma expander. Half of the animals were given a 2% DDFP emulsion, 0.7ml/kg i.e.14 μl i.v. DDFP/kg, and half of the animals received vehicle. The control rats died at 2.7 g hemoglobin (Hb)/100 ml and the control pigs at 3.0 g Hb/100 ml, while all test animals receiving DDFP survived at Hb levels of 1.4 (rats) and 2.0 g/100 ml (pigs) until terminated after having lived 2-4 hours longer than the control animals. One series of rats that was allowed to wake up from anesthesia exhibited normal behavior, walking, eating drinking and grooming. All test animals had normal blood pressure. After retransfusion of shed blood, the rats were kept for 21 days, after which they were sacrificed and multi-organ histology was performed. No changes were found in any specimen. To demonstrate the general applicability of gas transport by intravascular microbubbles, we tested the rate of whole body nitrogen washout in anesthetized, spontaneously breathing pigs treated with DDFP emulsion. The treatment pigs received DDFP emulsion, 0.1ml/kg i.v. and controls were given vehicle during the first 30 min of the 120 min long oxygen breathing session. The DDFP treated pigs eliminated the same amount of nitrogen in 68 min as the control animals did in 120 min with identical cardiac output, i.e. the washout time was reduced by 43%. Conclusion: These experiments demonstrate that both oxygen and nitrogen can be transported effectively by intravascular volume-stabilized DDFP microbubbles. If the results apply to humans, one can predict that the oxygen consumption of an oxygen breathing resting 75 kg person can be provided for by a total dose of 1.1 ml of dodecafluoropentane injected intravenously in form of an emulsion.

-1-

THE APPLICATION OF cDNA MICROARRAYS TO THE PROBLEM OF HEMORRHAGE/SHOCK

P. Bowman, J.L. Sondeen, B. Zhao, M.A. Dubick

The sequencing of human and other genomes is providing unprecedented opportunities for obtaining a comprehensive understanding of the genetic basis of disease and injury, and providing new targets for drug intervention that promise to revolutionize medicine. Functional genomics is providing global techniques to assess gene function by making use of information and reagents based on structural genomics. cDNA microarrays are one result of these functional genomic applications that we are applying to the problem of hemorrhage/shock by screening 15 tissues in the rat subjected to hermorrhage. Thus far, about 100 genes out of 5100 are significantly up or down regulated in lung, intestine, liver, and kidney as a function of time up to 24 hours following a severe hemorrhage, (40% removal of blood over 10 minutes) in a conscious, unresuscitated rat. Some of the genes are tissue specific while others are more generally expressed. We have begun to examine the effect of lactated Ringer's resuscitation on the pattern of gene expression and fluid resuscitation does not change the pattern of expression but accelerated the return to normal. We hypothesize that supplementation with appropriate compounds (glutamine, arginine, pyruvate) will depress the pattern of altered gene expression as affected tissues are supplied appropriate metabolic substrates.

-2-

RESUSCITATION OF THE MICROCIRCULATION IN SHOCK USING ORTHOGONAL POLARIZATION SPECTRAL IMAGING.

R.W. Barbee, R.N. Pittman, K.R. Ward, B.D. Spiess, R.R. Ivatury, C. Ince

Useful endpoints are needed to both diagnose the need for resuscitation and to ensure the adequacy of such efforts for the victim of trauma. The use of traditional clinical signs is often inadequate, and even the use of global endpoints such as lactate can fail to reveal subclinical shock states, organ specific ischemia and incomplete resuscitation. Imaging specific microcirculatory parameters could theoretically provide useful diagnostic and resuscitation endpoints. This has previously been clinically possible only with restricted beds such as the nailfold or bulbar conjunctiva. Orthogonal polarization spectral (OPS) imaging allows for direct and noninvasive visualization of the microcirculation from any mucosal surface by eliminating the need for transillumination or fluorescent dyes (1,2). It has been implemented in a hand held microscope (2), validated (3) and has allowed for the first time imaging of the microcirculation of human organs in health and disease (5,6). Coupled with appropriate software, the device is capable of measuring vessel diameter, red blood cell velocity, and functional capillary density. It is conceivable that detectable changes in these parameters may occur significantly earlier than changes in vital signs or in currently measured biochemical end-points such as lactate or carbon dioxide. It is also possible that restoration of these variables occur later, once resuscitation of

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